Edman sequencing and MADLI-TOF mass spectrometry. Percent hydrolysis of the peptide substrate was calculated by comparing the integrated peak areas for the two product peptides and the starting material derived from the absorbance at 214 nm. The specificity of the protease cleavage reaction was determined by performing the β-secretase assay in the presence of a cocktail of protease inhibitors (8 μM pepstatin A, 10 μM leupeptin, 10 μM E64, and 5 mM EDTA).

An alternative β-secretase assay utilizes internally quenched fluorescent substrates to monitor enzyme activity using fluorescence spectroscopy in a single sample or multiwell format. Each reaction contained 50 mM Na-MES (pH 5.5), peptide substrate MCA-EVKMDAEF[K-DNP] (BioSource International) (50 μM) and purified Hu-Asp-2 enzyme These components were equilibrated to 37 °C for various times and the reaction initiated by addition of substrate. Excitation was performed at 330 nm and the reaction kinetics were monitored by measuring the fluorescence emission at 390 nm. To detect compounds that modulate Hu-Asp-2 activity, the test compounds were added during the preincubation phase of the reaction and the kinetics of the reaction monitored as described above. Activators are scored as compounds that increase the rate of appearance of fluorescence while inhibitors decrease the rate of appearance of fluorescence.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention.

The entire disclosure of all publications cited herein are hereby incorporated by reference.

Claims

Article 34

AMENDED CLAIMS

[received by the International Bureau on 2 October 2000 (02.10.00);
original claims 1-141 replaced by new claims 1-150 (18 pages)]

1. A purified polypeptide comprising a mammalian Asp2 polypeptide that cleaves a mammalian β-amyloid precursor protein (APP), or a fragment, analog, or derivative of said mammalian Asp2 polypeptide that retains the APP cleaving activity.

2. A purified polypeptide according to claim 1, selected from the group consisting of:

(a) a polypeptide comprising a purified human Asp2(a) amino acid sequence set forth in SEQ ID NO: 4 or a fragment thereof that cleaves APP;

(b) a polypeptide comprising a purified human Asp2(b) amino acid sequence set forth in SEQ ID NO: 6 or a fragment thereof that cleaves APP;

(c) a polypeptide comprising the murine Asp2 amino acid sequence set forth in SEQ ID NO: 8, or a fragment thereof that cleaves APP;

(d) a polypeptide comprising a purified polypeptide having an amino acid sequence that is at least 95% identical to (a), (b), or (c).

3. A purified polypeptide according to claim 1, comprising a purified human Asp2(a) amino acid sequence set forth in SEQ ID NO: 4 or a fragment thereof that cleaves APP.

4. A purified polypeptide according to claim 1, said polypeptide comprising a portion of the human Asp2(a) amino acid sequence set forth in SEQ ID NO: 4, said portion including amino acids 22-501 of SEQ ID NO: 4 and lacking amino acids 1-21.

5. A purified polypeptide according to claim 1, said polypeptide comprising a portion of the human Asp2(a) amino acid sequence set forth in SEQ ID NO: 4 effective to cleave APP, said polypeptide lacking transmembrane domain amino acid residues 455-477 of SEQ ID NO: 4.

6. A polypeptide according to claim 5, said polypeptide lacking amino acids 454-501 of SEQ ID NO: 4.

7. A purified polypeptide according to claim 1, comprising a purified human Asp2(b) amino acid sequence set forth in SEQ ID NO: 6 or a fragment thereof that cleaves APP.

8. A purified polypeptide according to claim 1, said polypeptide comprising a portion of the human Asp2(b) amino acid sequence set forth in SEQ ID NO: 6, said portion including amino acids 22-476 of SEQ ID NO: 6 and lacking amino acids 1-21.